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SCIENTIFIC RESEARCH ON TWO ISOLATED *Vipera ursinii moldavica* POPULATIONS OF THE ROMANIAN BLACK SEA COAST: PRELIMINARY RESULTS

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ABSTRACT

During our field research, in the autumn of 2002 and 2003, we managed to examine 44 meadow vipers (*Vipera ursinii*) from two different populations along the Romanian Black Sea coast. We examined 29 animals from populations near Sfântu Gheorghe and 15 individuals from Periteasca, of which 20 were males (45.5 %) and 24 were females (54.5 %). Twelve of the observed animals were born those years (27.3 %), 8 of them were subadults (18.2 %) and 24 were adults (54.5 %). The presence of all age-groups suggested healthy populations. Finding one animal took in Sfântu Gheorghe 6^{50} field-hours, per person on average in 2002 and 2^{40} field-hours in 2003, while in Periteasca it took 1^{12} field-hours. This divergence can suggest difference in population size or abundance between the two populations. We recorded several morphometric data on every animal and we collected blood-samples from 22 adult

individuals (from caudal vein). Thus we had 15 samples from Sfântu Gheorghe (7 males, 8 females) and 7 from Periteasca (2 males, 5 females) at the beginning of genetic research.
Preliminary results are provided on the investigated morphological characters. Results are in accordance with data provided by earlier studies. When comparing the two populations, generally the differences seem to be minor, although we described some characters in which we found slight differences. In the future we will focus on these characters. Data are comparative for latter studies to detect negative tendencies as early as possible in order to preserve these populations for future generations.

KEY WORDS: viper, *Vipera ursinii moldavica*, Black Sea, coastal populations, morphological characters

INTRODUCTION

Meadow viper (*Vipera ursinii*) populations throughout Europe are declining. During the 20th century several of them became extinct. This so called *Vipera ursinii* complex consists of several subspecies and some recently re-evaluated species (JOGER *et al.*, 1992; NILSON and ANDREN, 2001) that are occurring in small and fragmented populations. A very sad example is the Hungarian meadow viper (*Vipera ursinii rakosiensis*) which was first described in 1893 (MÉHELY, 1893) with frequent occurrence in the Carpathian basin (from Vienna to Cluj) and by the end of the century became one of the most endangered (sub)species of the European fauna (KORS□S, 1991). When scientists realised the problem they also realised the lack of information on the species, making more difficult finding any way to stop decline.

In the Romanian Danube Delta and the Black Sea coast there are known populations of meadow vipers (TÖRÖK, 2002). These populations were earlier considered as *Vipera ursinii macrops* (CALINESCU, 1931), *Vipera (ursinii) renardi* (IONESCU, 1968; FUHN, 1969; FUHN and VANCEA, 1960; KISS, 1985) and recently described as *Vipera ursinii moldavica* (NILSON *et al.*, 1993; NILSON and ANDRÉN, 2001). There were some studies involving these populations (KORS□S *et al.*, 1997; NILSON and ANDRÉN, 2001) but sample sizes with the origin of these populations were rather small. Our study targeted these populations to provide more data on them for conservation purposes and for science.

STUDY SITES AND METHODS

Our first study area is on the Romanian Black Sea coast, on Grindul Saraturi approximately 7 km from the small village Sfântu Gheorghe. The site is bordered from the east by small sand dunes built by the Black Sea, while on the south-western part planted poplar (*Populus alba*) forests limit the

distribution of vipers. The habitat is mainly xero-thermophilous meadows on marine sandy ground (with many shell-fragments like *Mytilus galloprovincialis, Cardium* spp. and *Mya arenaria*) with characteristic plants as *Juncus maritimus limonium, Euphorbia, Salicornia*. The changing abundance of segdge (*Juncus maritimus*) creates a variable pattern: from lonely plants in wide areas to dense bushy-like parts with no glades, creating ideal basking places with safe hiding shelter in the vicinity. The lowest parts or former pools are occupied by the halophyl *Salicornia herbacea*. Further from the Black Sea shore small sandy hills divide the stripes of *Juncus*. On the side of the hills and dunes bushes are formed mainly by *Hippophae rhamnoides* and *Elaeagnus angustifolia* but other species like *Tamaris ramosissima, Robinia pseudacacia* can also be found. The top of the dunes is occupied by *Convolvulus parsicua*.

Orthopterans serving as prey items for vipers were found, with characteristic species like *Acrida hungarica, Mantis religiosa* and *Calliptamus barbarus*. Lizards like *Lacerta agilis euxinica* and *Eremias deserti* were also abundant on certain parts of the area. We also found *Natrix natrix natrix* both in melanistic and normal coloration on the site. From the potential aerial predators for the vipers of the zone (KISS *et al.*, 1997) we observed grey heron (*Aqrdea cinerea*), marsh harrier (*Circus aeruginosus*), short-toed eagle (*Circaetus gallicus*), pheasant (*Phasianus colchicus*), roller (*Coracias garrulus*), red-backed shrike (*Lanius collurio*), lesser grey shrike (*Lanius minor*), magpie (*Pica pica*) and hooded crow (*Corvus cornix*). As the area lies between the sea-shore and canals, serving as orientation points for migrating birds, we can assume large temporary presence of other bird species. The large number of injuries found on the top of the head and on the side of the body support our theory. Although we have not found any fresh digging, the bone remains of wild boar found near the site point to the presence of the species. Permanent human presence is not typical in the area, but extensively bred domestic animals are present all the time. These are mainly cattle and horse, which can pose a threat by their tramping and disturbance, but in the period of our study their numbers were limited. Other agricultural activity is not present in the area, as the soil is sandy and salty that is not favourable for other activities.

The other site is on Grindul Perisor, close to the fishing houses of Periteasca, 43 km away from the Sfântu Gheorghe site. Beside the pure distance the main obstacle between the two populations is the Sfântu Gheorghe branch of the river Danube, isolating them totally from each-other. Grindul Perisor is bordered by the Black Sea on the south-east, Canal Perisor on the north, Razim Lake on the north-western side and Periteasca Canal on the south. The canal that actually cuts rectangularly to the sea ends there leaving only a 50 m connection between the two parts of this area. On this area there is a pair of rails formerly used to eject boats to the sea. According to the fishermen the vipers use this area to move between the two sides and they even bask on concrete flats.

Vegetation and main habitat is quite similar to thet at Sfântu Gheorghe site. It is a mosaic of higro-halophylous and xero-thermophylous meadows. There are fewer elevations and the site is flatter. The marine dunes on the shore are build up mainly by fragments of marine shells, creating

white slopes with warmer microclimate that are perfect for basking even in cloudy periods. The vegetation is mainly formed by sedge, creating denser and sparser parallel stripes. Bushes are fewer on the this study site, mainly formed by *Hippophaë rhamnoides* and *Elaeagnus angustifolia*.

Although human presence is permanent, area distributed by vipers is not used intensively. There are extensively bred cattle and sheep, but in the period of observation it seemed fewer in numbers than as Sfântu Gheorghe. We found some digging and bone remains of wild boar. The permanent or temporary presence of aerial predators listed earlier are also very probable.

At Sfântu Gheorghe between 24 and 26 September, 2002 and 8 and 10 September, 2003, while at Periteasca between 28 September and 1 October 2002, we were systematically searching for vipers on the study sites. During search 6 persons in line, 2 to 3 m from each-other, were walking back and forth a 1.5 km stretch of each area. Place of discovery was marked and GPS data were recorded for each viper. Vipers were observed in the nearby camp and later released on the same place where they were previously found. Pictures were taken on the top, sides of head, throat and whole body, for later analysis and identification. Weight, lenth of body, caudal length were measured. After determining sex and probable age, every animal was given an identification number and their morphological data were recorded on a register sheet. Num,ber of the following plates was counted and recorded: Preventrale, Ventrale, Subcaudale, Sublabiale and Supralabiale on both sides, Anterior dorsal scale rows (one head length posterior from head), Mid-body and Posterior dorsal scale rows (one head length anterior to anal). State of shedding, pregnancy, injuries and deformities were also recorded. Blood samples were taken from the caudal vein of adult individuals for later genetic analysis. Later from photos we also counted the following scale numbers: Apicale, Canthale and Intercanthale and Intersupraoculare on both sides (between Frontale and Supraoculare), Circumoculare and Lorealia on both sides. Morphological asymmetry, as an indicator of homozygosity (SOULÉ, 1979), was calculated on certain characters and documented for analysis. We also counted the number of windings or blotches in dorsal zigzag band, and determined whether Parietal, Frontal or Nasal scales were divided.

RESULTS

At Sfântu Gheorghe site 12 vipers in 2002 and 17 vipers in 2003 were observed (Table 1). There was no recapture so far. In 2002 on average it took 6^{50} hours for one person to find a viper, while in 2003 it took 2^{40} hours. In 2002 five specimen were born that year while in 2003 we have not found any young. Two females were gravid suggesting that our slightly earlier visit probably preceded birth. Two vipers were subadult in 2002 and three in 2003. From males the smallest was 216 mm long (29 mm tail) weighed 7.5 g, while the biggest was 535 mm long (71 mm tail) and weighed 68.0 g. The smallest female was 187 mm long (21 mm tail) and weighed 5.0 g, while the

biggest was 502 mm long (43 mm tail) and weighed 72.0 g (there was a 449 mm long female weighing 76.0 g, with clearly visible signs of a recent meal).

Table 1

Number of observed and examined vipers
according to their sex and place of observation

Sample locality	**Sample size**		
	male	**female**	**total**
Sfântu Gheorghe 2002	5	7	12
Sfântu Gheorghe 2003	8	9	17
Sfântu Gheorghe total	13	16	29
Periteasca total	7	8	15
Total	**20**	**24**	**44**

At Periteasca in 2002, 15 vipers were examined (Table 1). On average it took 1^{12} hour per person to find a viper. (Bad weather cut short our field-study in 2002 and in 2003 due to the same problem we were unable to approach the site.) We located two animals south of Periteasca-canal suggesting that viper distribution can even reach further to Portitza as the same

Table 2

Average weight, total length, tail length and snout-vent-length (SVL) of different age-groups of the observed animals (Mean □ SD, Minimum-Maximum).
All male and female tail length proved statistically different (F-test), while total lengths and SVL-s only in the 2-winter group, therefore we averaged them separately.

	Age groups							
(N)	**0 winter (12)**		**1 winter (8)**		**2 winter (15)**		**3(+) winter (9)**	
Body weight (g)	6.6 □ 1.7 4-10		25.0 □ 8.4 11-38		39.1 □ 13.2 25-76		58.2 □ 11.2 46-73	
Total length (mm) Male Female	203.2 □ 13.6 182-222		348.9 □ 18.0 315-372		396.6□27.7 365-441	429.6□26.3 388-451	493.1 □ 18.4 471-535	
Tail length (mm) Male Female	28.3□2.7 24-32	21.0□2.6 16-23	49.0□1.7 47-50	34.6□2.6 31-38	53.7□5.9 46-62	43.8□4.7 36-50	65.3□4.0 62-71	47.8□3.6 43-53
SVL (mm) Male Female	178.5 □ 10.4 165- 191		308.9 □ 18.8 284 - 334		342.9 □27.2 319-384	385.9 □ 23.6 347-409	437.6 □ 16.3 414 - 464	

habitat continues that way. Seven vipers were born that year and another three were subadults. The smallest male was 192 mm long (27 mm tail) and weighed 5.0 g, while the biggest was 498 mm long (62 mm tail) and weighed 51.0 g. The smallest female was 182 mm long (16 mm tail) and weighed 4.0 g, while the longest was 592 mm long (48 mm tail) and weighed 48.0 g, thus the heaviest weighed 50.0 g with 481 mm length (53 mm tail).

We managed to gain blood samples from 15 individuals at Sfântu Gheorghe (7 males, 8 females) and 7 individuals at Periteasca (2 males, 5 females). Results from the analysis of similarity using randomly amplified polymorphic DNA fragments (RAPD) is under-way, and will be published later.

During statistical analysis of raw data we compared populations, sexes and age-groups for all recorded morphological characters (Scheffé's F-tests). We only pooled those groups where there was no statistically significant diffrenece between them. Certainly weight and total length differed between age-groups. Tail length differed between sexes and age-groups as well. The difference between sexes was significant only in the 2-winter group when we compared snout-vent lengths, that is total length reduced by tail length (Table 2).

Generally in the analysis of scale numbers we were more interested in the comparison of the two population. There were no differences in the averages of number of Preventral plates, Ventral plates, Subcaudal plates, Dorsal scale rows, Canthal scales, Intercanthal scales. We found some small differences between the two populations in Supralabial, Sublabial, Loreal and Intersupraocular scale numbers (Table 3). The Subcaudal plate number is a character where sexes distinctly differ from each-other. We also found some slight differences between sexes in Intercanthal plate numbers and also in the number of windings or blotches in the dorsal zigzag band. Males from Periteasca differed significantly from the others in two characters. Average number of Circumocular scale (sum of two sides) were slightly lower than the average (17.7 □ 1.6 with a range between 15-20). The average number of Apical plates were slightly higher (1.7 □ 0.5 with a range between 1-2). Five (71 %) out of 7 males from Periteasca had 2 Apicals while all of the 8 females had single. In Sfântu Gheorghe two (15 %) males out of 13 and two (13 %) females out of 15 had double Apicals. Three (two young and a subadult) out of the five males with souble Apical were found within 100 m and another young was found within 500 m suggesting that at least the three with same age can be related to each-other, perhaps over representing this character. All females from Periteasca showed posterior dorsal scale row reduction (from 21 to 19 rows). Dorsal scale row reduction from 21 to 19 occurred in the first part of three (43 %) males from Periteasca and of seven (47 %) females and six (46 %) males from Sfântu Gheorghe. Thus 12 animals (80 %) from Periteasca and 16 (55 %) individuals from Sfântu Gheorghe showed posterior reduction.

Table 3

Averages of the examined morphological characters
(Mean □ SD, Minimum-Maximum).
Only significantly different (F-test) groups are not pooled.

	Total (N = 44)	
Preventral plates	1.6 □ 0.7 0-3	
Ventral plates	140.5 □ 4.2 130-152	
Anterior dorsal scale rows	21.8 □ 0.8 21-23	
Mid-body dorsal scale rows	20.2 □ 1.0 18-22	
Posterior dorsal scale rows	16.9 □ 0.5 15-18	
Canthal scales *	3.9 □ 0.3 2-4	
Circumocular scales *	19.0 □ 1.4 14-22	
	Sf.Gheorghe (N = 29)	**Periteasca (N = 15)**
Apical plates	1.2 □ 0.4 1-2	1.3 □ 0.5 1-2
Intersupraocular scales *	6.0 □ 2.2 2-11	3.6 □ 1.5 2-8
Supralabial scales *	19.0 □ 1.4 17-21	18.1 □ 1.4 16-20
Sublabial scales *	20.4 □ 1.3 19-23	21.3 □ 1.4 19-24
Loreal scales *	10.5 □ 2.2 8-17	8.6 □ 1.5 5-11
	Male (N = 20)	**Female (N = 24**
Subcaudal plates	38.3 □ 1.7 35-40	29.0 □ 1.5 26-32
Intercanthal scales	8.5 □ 2.0 3-11	7.3 □ 2.3 4-12
Number of windings or blotches in dorsal zigzag band	73.8 □ 6.3 65-90	68.9 □ 5.0 62-81

* sum of both sides

Only one female from Sfântu Gheorghe had divided Parietal plates. No divided Frontal plate was observed. Partial division of Nasal plates were observed on several animals. One male and one

female had partially divided Nasal plate only on one side (both from Sf#ntu Gheorghe). Five (33 %) females from Sfântu Gheorghe and three (38 %) females from Periteasca had partially divided Nasal plate on both side while no males showed this phenomenon.

Asymmetry of Supralabials, Sublabials, Circumoculars, Loreals, Canthals and Intersupraoculars were investigated (Table 4). There was only one individual (male from Periteasca) with total symmetry in these characters. Asymmetry between Canthals is very rare, only one female from Sfântu Gheorghe showed this phenomenon. There was only one major difference between the two populations: the asymmetry in Supralabials.

Four specimen had injuries on the head, two on the side of the body and one on the tail. Out of the seven (15.9 %) recorded injuries two (13.3 %) were from Periteasca and five (17.2 %) from Sfântu Gheorghe, while two (10 %) were males and five (20.8 %) were females.

DISCUSSION

Results are preliminary, meaning that we would like to enlarge sample sizes in the future. For example so far we have no recapture data helping in the estimation of population sizes. By comparing the average required time of finding one viper, Periteasca population seems larger (or vipers are more abundant at the study site than on Sfântu Gheorghe). Levels of human related disturbance is surely lower on the more isolated Grindul Perisor, maybe vipers at Sfântu Gheorghe are more sensitive to disturbance. There was no real difference in number of injuries suggesting an equal predation pressure in both places. The fact that injuries were nearly double on females than on males can be the outcome of more time spent basking by females during pregnancy or greater survivor of attacks, although the fairly equal sex ratio did not support the latter theory. Sex ratio did not differed between the two populations.

There was no difference in weight and length between the age-groups of the two population, suggesting no difference in habitat circumstances. Generally the averages of studied morphological characters did not differed considerably from earlier published results (NILSON and ANDRÉN, 2001). There were five morphological characters were difference was shown between vipers of the two sites. The result suggests population specific qualities that latter investigation can focus on. Results of genetic analysis will hopefully provide more details on this issue. Percentage of asymmetry did not differed from data of other researchers (NILSON and ANDRÉN, 2001). The very low asymmetry of Supralabials (and the lower percentages in three other characters) in vipers from Sfântu Gheorghe can suggest a higher heterozygosity level in this population, but it is too early to consider it as significant difference.

Table 4

Percentages of asymmetry in examined bilateral morphological characters

	Sfântu Gheorghe (N = 29)	**Periteasca (N = 14)**	**Total (N = 43)**
Supralabial scales	13.3	75.9	54.5
Sublabial scales	60.0	79.3	72.7
Circumocular scales	53.3	58.6	56.8
Loreal scales	33.4	48.3	43.2
Canthal scales	3.5	0.0	2.3
Intersupraocular scales	60.0	44.8	50.0
Total high	**5**	**4**	**5**
Total low	**1**	**0**	**0**

Generally our first priority to provide more data on these populations has succeeded. These two populations of this unique snake are very important, not only at national level but at European one too. As conservation efforts on other populations of this or related species have difficulties in maintaining populations or stopping their extinction, the importance of the Danube Delta populations will rise. These populations can be preserved for future generations through conservation of their habitat. The conservation provided by the Danube Delta Biosphere Reserve can guarantee the long-term existence of these populations and our data can serve as comparison for latter studies to detect negative tendencies as early as possible.

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